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Calcium Fortification of Bolu Cukke (Traditional Cakes of South Sulawesi, Indonesia) Using Threadfin Bream Fish Bone

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ABSTRACT

The fishery industry generates substantial fish bone waste, which remains underutilized despite its high calcium content. This study aimed to investigate the potential of threadfin bream (Nemipterus nematophorus) fish bones as a sustainable calcium source for fortifying bolo cokes, a traditional cake from South Sulawesi, to enhance its nutritional value while promoting the utilization of fishery by-products. Calcium was extracted from fish bones using bromelain, an enzyme derived from pineapple peels. The extracted calcium-rich powder was then used to replace part of the premix flour (10%, 20%, 30%) in bolo cokes preparation, with varying levels of tiramisu flavouring (15%, 30%, 45%). The experiment followed a Completely Randomized Design with three replications for each treatment. Physicochemical parameters, including moisture, ash, carbohydrate, calcium content, and organoleptic attributes (colour, aroma, texture, and taste), were evaluated. The enzymatic extraction process yielded a high calcium content with a recovery rate of 43.8%, surpassing the Indonesian National Standard. Fortification significantly increased the ash and calcium content of bolu cokes, with the highest substitution and flavouring levels producing cakes that maintained acceptable sensory qualities, including favourable colour, aroma, texture, and taste. Threadfin bream fish bones are a promising and sustainable source of calcium for bakery product fortification.

1-Introduction

Fishery industry waste, particularly fish bones, represents a significant yet underutilized resource, accounting for about 14% of total fish composition and offering a rich source of calcium, an essential mineral crucial for bone health and physiological function [1] . Inadequate calcium intake is linked to serious health issues such as osteoporosis and stunted growth, highlighting the importance of accessible calcium sources for public health [2]. While fish bones have been recognised as a potential natural calcium supplement, their application in mainstream food fortification remains limited, and most fish bone waste continues to be discarded rather than processed for nutritional use [3,4].

Previous research has explored fish bone-derived calcium in food fortification, demonstrating its effectiveness in increasing calcium content and supporting bone health in various food products [5–7]. However, these studies have not extensively addressed the incorporation of calcium-rich fish bone powder into traditional foods such as bolu cukke, a popular cake from South Sulawesi made primarily from rice flour and brown sugar. Bolu cukke is typically low in calcium, making it an ideal candidate for fortification to enhance its nutritional value without compromising its authentic taste and texture. Thus, there is a clear need for research that utilises fishery by-products and adapts traditional recipes to meet modern nutritional demands.

The present study aims to fill this gap by evaluating the impact of fortifying bolu cukke with calcium extracted from Threadfin Bream (*Nemipterus nematophorus)* fish bones. Calcium fortification of Bolu Cokes, a traditional cake from South Sulawesi, Indonesia, using threadfin bream fish bone is hypothesized to enhance the nutritional value of the product without compromising its sensory characteristics. By incorporating finely processed fish bone as a natural calcium source, this approach aims to address calcium deficiency in local diets while maintaining the cultural and culinary integrity of the cake.The research explicitly assesses the effects on nutritional content and organoleptic properties taste, aroma, and texture of the fortified cake. By incorporating fishery waste into a culturally significant food product, this study offers a novel approach to addressing calcium deficiency while promoting sustainable resource use. The findings are expected to contribute to diversifying fishery product applications and developing functional foods that combine tradition with improved nutrition.

2-MATERIALS AND TOOLS

Research Materials and Tools

This study utilized various materials and equipment to investigate calcium extraction from Threadfin Bream fish bones and its fortification in Bolu Cukke cake premix flour. The primary raw materials included Threadfin Bream fish bones, which were sourced fresh and cleaned thoroughly to remove residual meat. Pineapple peels from young fruits were used to extract crude bromelain enzyme, facilitating hydrolysis. Additional ingredients included analytical-grade sodium chloride (NaCl), distilled water, commercial wheat flour, powdered sugar, baking soda, and food-grade tiramisu powder for flavouring.

For chemical analyses, reagents such as silver nitrate ($AgNO_3$), sulfuric acid (H_2SO_4), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), boric acid (H_3BO_3) at 4 % concentration, hydrochloric acid (HCl) at 0.2 N, potassium permanganate ($KMnO_4$) at 0.01 N, and bromocresol green indicator were employed. All chemicals were of analytical grade and procured from Merck, Germany.

The equipment used in this research included a KitchenAid mixer (Model KSM150PS, USA) for mixing ingredients, a Memmert

UN55 oven (Germany) with precise temperature control (±1°C) for drying and roasting processes, and a Tuttnauer 3870EL autoclave set at 121°C and 15 psi for bone softening. A digital scale (Ohaus Pioneer PA214, USA) with 0.01 g precision was used for accurate weighing. Bone powder was produced using a custom-made stainless steel bone crusher. Additional tools included stainless steel knives and spoons, aluminum cake molds (20 cm diameter), gas stoves, food-grade basins and gutters, desiccators (Thermo Scientific, USA), porcelain crucibles, clamping tools, Kjeldahl flasks (Pyrex, USA), catalyst tablets, Erlenmeyer flasks, borosilicate goblet glasses, and Whatman No. 42 filter paper.

Research Procedure

Threadfin Bream Fish Bone Calcium Extraction

The calcium extraction procedure was adapted from Fitri et al. (2022) with modifications to optimise yield and purity. Initially, the fish bones were cleaned meticulously to remove all meat residues. Subsequently, 350 mL of distilled water was mixed with 10% (w/v) sodium chloride and crude bromelain enzyme extracted from young pineapple peels. The pH of this solution was carefully adjusted to 6.5 ± 0.1 using NaOH or HCl to ensure optimal enzymatic activity. The fish bones were then submerged in this solution and subjected to hydrolysis by heating at 60°C ± 1°C for 10 hours in a controlled water bath (Memmert WNB 14, Germany).

After hydrolysis, the bones were washed repeatedly with distilled water until the wash water reached a neutral pH (~7.0). The softened bones were autoclaved at 121°C and 15 psi for 3 hours to further soften the bone matrix. Following autoclaving, the bones were dried in an oven at 60°C ± 1°C for 24 hours. The dried bones were subsequently crushed into a fine micro calcium powder using the bone crusher.

Fortification of Calcium Bone of Threadfin Bream Fish in Bolu Cukke Premix Flour

Wheat flour was roasted in the Memmert UN55 oven at 90°C ± 2°C for 25 minutes to enhance flavor and reduce moisture content. After cooling to ambient temperature (25°C ± 2°C), 600 grams of the roasted flour was weighed for each treatment. The flour was then mixed with calcium bone powder at varying concentrations (10%, 20%, and 30% w/w) and tiramisu powder at three levels (15%, 30%, and 45% w/w), corresponding to the experimental design variables. 30% powdered sugar and 1.5% baking soda were added to this mixture. The mixture was thoroughly stirred and sifted through a 60-mesh sieve to ensure homogeneity and smooth texture.

Preparation Bolu Cukke

The cake batter was prepared by mixing the premix flour with baking powder (1.5% w/w) and salt (0.5% w/w), mixing the dry ingredients evenly. Fresh eggs and brown sugar were beaten at 300 rpm for 5 minutes using the KitchenAid mixer until the mixture became fluffy. Coconut milk (30% w/w) was then gradually incorporated into the egg mixture while stirring at low speed. The dry ingredient mixture was slowly folded into the wet mixture to avoid lumps. Water (approximately 300 mL per batch) was added to achieve a thick but pourable batter consistency. The batter was poured into buttered aluminium cake moulds and baked in the Memmert UN55 oven at 180°C ± 2°C for 30 to 40 minutes. Doneness was confirmed by the toothpick test, ensuring the cake was fully cooked.

Research Design

This study employs an experimental research design with a complete random design, utilizing two research variables. The first variable was the percentage of calcium in Threadfin Bream fish bones (A), and the second variable was the percentage of tiramisu seasoning (B). Each was repeated three times, resulting in 27 experimental units. The first variable was the percentage of calcium derivative in Threadfin Bream fish bone (A), divided into three treatments: A1 (10%), A2 (20%), and A3 (30%). The second variable is the percentage of tiramisu flavor (B), divided into three levels B1 (15%), B2 (30%), and B3 (45%).

Experimental Design

This study employed a two-factor Complete Randomized Design (CRD) with three levels for each factor. The first factor was the concentration of calcium bone powder (10%, 20%, and 30% w/w), while the second was tiramisu flavouring powder (15%, 30%, and 45% w/w). Each treatment combination was replicated three times, resulting in 27 experimental units.

Analytical Parameters

The characterization of the fortified Bolu Cukke included yield determination calculated as the ratio of final product weight to initial raw materials, following AOAC (2005) Guidelines. Proximate analyses were conducted to measure moisture content by oven drying at 105°C for 3 hours, ash by incineration in a muffle furnace at 550°C for 6 hours, and carbohydrate content by difference. Calcium content was quantified using Atomic Absorption Spectrometry (AAS) with a PerkinElmer AAnalyst 400 instrument (USA) at a wavelength of 422.7 nm. Samples were prepared via wet ashing digestion using nitric acid and hydrogen peroxide

Functional group characterization of the calcium powder was performed using Fourier Transform Infrared Spectroscopy (FTIR) with a Thermo Scientific Nicolet iS10 spectrometer (USA). Spectra were recorded over the range of 4000 to 400 cm^{-1}, with 32 scans per sample and a resolution of 4 cm^{-1}, as described by [10]

Data Analysis

Statistical analyses were performed using IBM SPSS Statistics version 27.0 for Windows. A two-way Analysis of Variance (ANOVA) was conducted to assess the effects of calcium concentration and tiramisu flavor level on the measured parameters. The homogeneity of variances was verified using Levene's test. When significant differences were detected at a 5% significance level ($\alpha = 0.05$), Duncan's Multiple Range Test (DMRT) was applied for post hoc comparisons to identify differences between treatment means. Results were reported as means ± standard deviations.

3-RESULTS AND DISCUSSION

Characteristics of Threadfin Bream Fish Bone Calcium

The calcium yield obtained from the enzymatic extraction of Threadfin Bream fish bones in this study was 43.8%, which is considerably higher than yields reported for other fish species using different extraction methods, such as catfish (27.25% via citric acid hydrolysis) [11], tuna (24.5% via alkaline treatment) [12], and mackerel (25.6% via high-speed pulverization) [13] This elevated yield demonstrates the effectiveness of the bromelain enzyme derived from pineapple peel in hydrolysing the protein matrix that encapsulates bone minerals. The enzymatic process facilitates a more efficient release of calcium ions without compromising mineral integrity. The enzymatic process contrasts with conventional thermal methods, often resulting in mineral loss due to dissolution or degradation during heating [14,15].

Table 1. Characteristics of Calcium Powder from Threadfin Bream Fish Bones

Characteristics	Value	unit	Standard of Fish Flour (SNI 01 – 3158 – 1992)
Yield	43.8	%	-
Moisture Content	4.5	%	8

Calcium Content	28.5	%	20

The calcium content of the extracted powder was measured at 28.5% , exceeding the Indonesian National Standard (SNI 01-3158-1992) minimum requirement of 20% for calcium in bone powder intended for food applications. This elevated calcium concentration enhances the product's nutritional value, making it a promising candidate for food fortification and dietary supplementation. The higher calcium content relative to other studies may be attributed to the optimized enzymatic hydrolysis conditions and the intrinsic mineral composition of Threadfin Bream bones. Notably, the calcium powder's moisture content was 4.5%, well below the SNI maximum limit of 8%, which is critical for ensuring product stability, reducing microbial growth risk, and extending shelf life. Compared to other fish bone powders, such as catfish (10.35%) (Nuramaniyah & Nurul, 2021) and snapper (8.40%) [17]. The lower moisture content further indicates the efficacy of the post-extraction drying process.

The extraction method significantly impacts the calcium content obtained from the bones of the Threadfin Bream fish. Various techniques, such as heat treatment [18], ultrasonication [19], and nano milling [20], have been explored to optimize the extraction of calcium from fish bones. The yield of calcium extraction (43.8%) represents a significant improvement over other reported methods, reflecting the enzymatic approach's capacity to maximize mineral recovery. This is mechanistically supported by bromelain's proteolytic action, which selectively cleaves peptide bonds in the protein matrix surrounding hydroxyapatite crystals. This enzymatic degradation exposes the mineral phase, enabling efficient calcium release while preserving the hydroxyapatite structure, as confirmed by FTIR analysis.

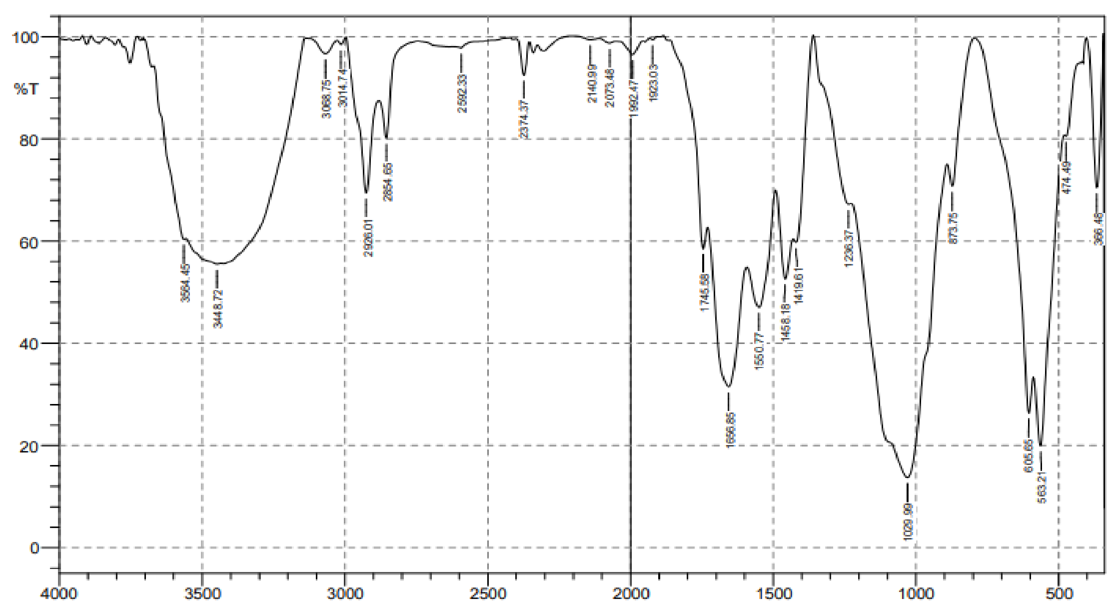

Figure 1. FTIR analysis of threadfin bream bone calcium powder

Fourier Transform Infrared Spectroscopy (FTIR) analysis of the calcium powder revealed distinct absorption peaks characteristic of hydroxyapatite, the primary mineral constituent of fish bone. Strong absorption bands observed between 1030 and

1100 cm⁻¹ correspond to asymmetric stretching vibrations of phosphate groups (PO_4^{3-}) [21] confirming the presence of the mineral phase. Additional peaks at approximately 600 cm¹ and 560 cm¹ are attributed to bending vibrations of phosphate groups, further substantiating the retention of the mineral structure post-extraction. A broad absorption peak around 3400 cm^{-1} indicates hydroxyl (-OH) group vibrations, signifying that the hydroxyl component of hydroxyapatite remains intact. This preservation of functional groups is critical for maintaining calcium's bioavailability and functional properties in food applications.

Importantly, the FTIR spectrum showed minimal absorption peaks related to organic compounds such as proteins and lipids, indicating that the enzymatic process effectively removed the organic matrix without damaging the mineral phase [22]. This contrasts with thermal or chemical extraction methods, which often result in residual organic matter or mineral degradation. The sharp and clear phosphate peaks in the FTIR spectrum demonstrate that enzymatic extraction maintains the crystalline integrity of hydroxyapatite, which is essential for optimal calcium bioavailability and functionality.

The enzymatic extraction method employed in this study yields calcium powder from Threadfin Bream fish bones with high calcium content, low moisture, and superior mineral integrity as evidenced by FTIR analysis. These findings align with and extend previous literature, highlighting enzymatic hydrolysis as an exceptional, sustainable, and efficient technique for producing high-quality calcium supplements from fish byproducts. The enhanced yield and purity, combined with compliance with national standards, underscore the potential of this calcium powder for use in food fortification and nutritional supplementation.

Calcium Content of Bolu Cukke

Incorporating calcium into bakery products is increasingly recognized as an effective strategy to address dietary mineral deficiencies. In this study, calcium phosphate derived from Threadfin Bream (*Nemipterus* spp.) bones and tiramisu flavoring to bolu cukke premix significantly enhanced the calcium content, indicating practical potential and nutritional value.

The findings indicated that the calcium content in bolu cukke rose with the increased concentration of tiramisu and calcium derivatives from Threadfin Bream fish bones (Tabel 2). Statistically, the interactive influence of calcium concentration and tiramisu flavor addition was highly significant. The significance of these factors reflects not only the effectiveness of the calcium fortification strategy but also the compatibility of tiramisu flavor as a carrier that potentially contributes to mineral retention. The strong interaction effect suggests a synergistic mechanism where tiramisu components, possibly polyphenolic compounds or stabilizers, contribute to minimizing mineral degradation during baking.

Table 2. Characterization of the chemical of fortified bolu cukke fish bone calcium powder threadfin bream and tiramisu flavouring

Treatment	Calcium	Ash	Moisture	Carbohydrate
A1B1	8,55 ± 0,47^h	1,14 ± 0,12^c	8,4 ± 0,04^c	79,01 ± 0,51^c
A1B2	18,48 ± 0,39^f	1,66 ± 0,09^b	9,69 ± 0,41^b	80,67 ± 0,1^b
A1B3	27,41 ± 0,21^c	2,71± 0,04^a	10,5 ± 0,06^a	82,13 ± 0,57^a
A2B1	8,42 ± 0,29^h	1,25 ± 0,16^c	8,68 ± 0,03^c	80,4 ± 0,25^b
A2B2	19,56 ± 0,11^e	1,74 ± 0,17^b	9,62 ± 0,06^b	80,98 ± 0,41^b

A2B3	28,49 ± 0,16^{b}	2,63 ± 0,4^{a}	10,5 ± 0,04^{a}	82,2 ± 0,6^{a}
A3B1	9,34 ± 0,23^{g}	1,32 ± 0,07^{c}	8,48 ± 0,37^{c}	80,39 ± 0,44^{b}
A3B2	20,45 ± 0,12^{d}	1,74 ± 0,06^{b}	9,9 ± 0,01^{b}	80,64 ± 0,41^{b}
A3B3	29,69 ± 0,14^{a}	2,76 ± 0,18^{a}	10,8 ± 0,02^{a}	82,71 ± 0,04^{a}

From a compositional perspective, calcium from fish bone is primarily present as hydroxyapatite, a bioavailable and stable form of calcium. When finely processed into powder form, its small particle size allows for even distribution within the dough, enhancing both its solubility and retention during thermal exposure. This characteristic plays a crucial role in the consistent increase in calcium levels across treatments, confirming assertions made by Wijayanti et al., (2021) Regarding the critical role of particle size and pre-treatment in fish bone mineral utilization.

Unlike some studies that have reported a decrease in mineral bioavailability due to complexation with proteins or fibers during baking [24], the current findings do not indicate such antagonistic interactions. This discrepancy could be explained by the specific formulation used in this study, which likely minimizes anti-nutrient effects by proportioning ingredients.

This study's contribution to the formulation of calcium-rich functional bakery products offers a dual advantage: sensory appeal through tiramisu flavour and improved mineral nutrition through natural calcium fortification. This innovation aligns with current trends in food science that emphasise health-oriented product development using sustainable and underutilised resources, such as fish processing by-products.

Ash Content of Bolu Cukke

Ash content is a critical parameter in food quality assessment as it reflects the mineral composition of the product, which in turn contributes to its nutritional profile. This study evaluated the ash content in Bolu Cukke premix by adding calcium phosphate derived from threadfin bream fish bone flour and tiramisu flavouring.

The results of this study (Tabel 2) align with the theory that adding minerals, particularly calcium powder, increases the ash content in food products. Previous studies have found that using calcium from natural ingredients, such as fish bones, can significantly increase ash content in flour-based processed products [25]. A clear trend was observed in which the ash content varied significantly with the addition of tiramisu flavouring, while the impact of the fish bone-derived calcium phosphate was not statistically significant. This pattern suggests that the tiramisu flavoring was more dominant in influencing the ash content than the calcium phosphate supplementation alone. The interaction between calcium phosphate and tiramisu flavoring was also examined, but no significant interaction was observed. This suggests that the effects of each factor on ash content occurred independently, without synergistic or antagonistic interference. The increase in ash content associated with tiramisu flavoring could be attributed to the presence of inorganic compounds in the flavoring formulation, such as cocoa, coffee derivatives, or stabilizers, which may contribute trace minerals to the product. This observation aligns with emerging findings in food chemistry, where specific flavoring agents, though primarily used for sensory enhancement, also impact nutritional content due to their complex composition [26].

Incorporating flavoring agents and mineral additives may alter the physicochemical environment of the premix, potentially affecting moisture binding, thermal decomposition during ashing, and the retention or release of mineral components

[27]. This is particularly relevant in complex matrices like bolu cake flour, where protein-starch-fat interactions may influence the bioavailability and measurable concentration of minerals post-processing. Moreover, the absence of significant effects from calcium phosphate might be explained by the mineral compound's limited solubility or bioaccessibility under the processing conditions applied.

Moisture content of Bolu Cukke

The moisture content in food is a critical parameter affecting a food product's quality, texture, shelf life, and safety. Moisture content is the percentage of water in food, both bound and free. Water firmly bound in the food matrix is difficult to evaporate, whereas free water is more easily mobilized and plays a crucial role in microbial activity and chemical reactions. This study showed (Tabel 2) that the higher the addition of calcium powder from fish bones and tiramisu seasoning in premixed flour, the higher the moisture content in bolu cukke. Based on the results of statistical analysis, the addition of tiramisu flavor was proven to have a real and consistent effect on increasing the moisture content of the product. In contrast, the addition of calcium phosphate from kurisi fish bones and the interaction between the two factors did not show a significant change. The consistency of this tiramisu flavor effect was confirmed through follow-up analysis (Duncan test), where each group of tiramisu flavor treatments showed significant differences in moisture content. At the same time, the variation in calcium phosphate did not have a considerable impact. This confirms that in the formulation of bakery products such as bolu cukke, the characteristics of the flavoring ingredients greatly determine the main physical parameters, such as moisture content.

This phenomenon can be explained through the ingredients' properties, where calcium powder from fish bones tends to attract and retain moisture in the dough [28]. Additionally, the tiramisu seasoning added to the formulation can also influence the interaction between the dough's components and water, thereby increasing the moisture content in the final product. Minerals such as calcium can form bonds with other elements in food products, including proteins or carbohydrates. This can alter the product's microstructure and affect the moisture content.

Mechanistically, the increase in moisture content observed with tiramisu flavoring can be attributed to the hygroscopic properties of certain flavoring compounds, which can bind and retain water within the dough matrix [29]. The presence of minerals such as calcium, especially in the form of calcium phosphate, can theoretically interact with proteins and carbohydrates to form a network that retains water. However, in this study, the contribution of fish bone calcium phosphate to water retention was not statistically significant, possibly due to its concentration or its interaction with other dough components. Adding food additives to bakery products increases the moisture content due to their properties, which can bind water in the dough. Research by H. Liu et al. (2025) Has also demonstrated that using flavoring agents in dough can alter the water retention properties of bakery products.

Carbohydrate Content of Bolu Cukke

Carbohydrates are essential macronutrients in flour-based food products such as *bolu cukke*, serving as the principal energy source and significantly influencing structural and sensory properties. Calcium ions appear to contribute mechanistically to changes in carbohydrate content by altering starch behavior during processing. Prior research has established that calcium can bind with starch molecules, thereby modifying the gelatinization and retrogradation process [31]. In this research (Tabel 2), the calcium phosphate may enhance water retention during baking, promoting a more extensive gelatinization process and potentially increasing the measured carbohydrate availability. Furthermore, calcium may act as an inhibitor of starch-degrading enzymes such as amylases, contributing to reduced breakdown of starch and a subsequent increase in retained carbohydrates in the final product [32].

Tiramisu flavoring, commonly formulated with carbohydrate-rich carriers like maltodextrin, also markedly affects carbohydrate levels. The observed pattern suggests that increasing flavoring concentrations correspond with higher carbohydrate content. This finding aligns with the principles reported by [33], who highlighted the role of powdered flavors and carriers in elevating overall carbohydrate measurements in bakery products. While primarily added for sensory appeal, flavoring agents can significantly alter nutritional composition, an essential consideration for product development and nutritional labeling.

The statistical interaction between calcium bone flour and tiramisu flavoring underscores the complexity of ingredient interactions within composite food systems. Although both components individually contributed to increased carbohydrate content, their combined effects suggest more than simple additive behavior. It is plausible that mineral–carbohydrate interactions influenced the matrix structure, affecting diffusion, binding, or thermal behavior of starch during the baking process [34].

Interestingly, the effect of tiramisu flavoring appeared more dominant than calcium addition in elevating carbohydrate levels. This may reflect the direct carbohydrate contribution from flavoring compounds, compared to calcium's more indirect functional role. Nonetheless, the influence of calcium on starch dynamics should not be overlooked, especially given its broader implications on texture, water activity, and shelflife stability.

These findings are consistent with previous studies that emphasize the multidimensional role of ingredient interactions in determining nutritional profiles in processed food [35]. They also illustrate the importance of considering ingredient synergy, rather than isolated effects, when optimizing food formulations. Future work may further investigate underlying molecular interactions through techniques such as differential scanning calorimetry (DSC) or Fourier-transform infrared spectroscopy (FTIR) to provide deeper insights into complex matrices' starch–mineral–flavor interactions.

Organoleptic of Bolu Cukke

Organoleptic testing in food products is a sensory evaluation method that utilizes the human senses to assess the quality of a product based on its color, aroma, taste, and texture. This evaluation is crucial in the food industry because it provides insights into consumer preferences and product market acceptance. Factors such as raw materials, formulations, and processing processes can affect a product's organoleptic properties.

Table 3. Characterization of the organoleptic of fortified bolu cukke fish bone calcium powder threadfin bream and tiramisu flavouring

Treatment	Colour	Aroma	Texture	Taste
A_1B_1	$4,14 \pm 0,02^{ab}$	$3,92 \pm 0,04^{e}$	$3,7 \pm 0,06^{ab}$	$3,79 \pm 0,05^{c}$
A_1B_2	$4,17 \pm 0,05^{ab}$	$3,92 \pm 0,1^{e}$	$3,66 \pm 0,05^{ab}$	$3,81 \pm 0,06^{c}$
A_1B_3	$4,31 \pm 0,02^{a}$	$3,95 \pm 0,05^{e}$	$3,62 \pm 0,08^{b}$	$3,94 \pm 0,04^{c}$
A_2B_1	$3,95 \pm 0,29^{cd}$	$4,22 \pm 0,07^{d}$	$3,65 \pm 0,04^{ab}$	$4,02\pm 0,02^{c}$

A_2B_2	3,87 ± 0,03^{d}	4,2 ± 0,02^{d}	3,73 ± 0,02^{a}	4,14 ± 0,13^{bc}
A_2B_3	3,76 ± 0,05^{d}	4,27 ± 0,05^{d}	3,68 ± 0,05^{ab}	4,07 ± 0,27^{bc}
A_3B_1	4,06 ± 0,04^{bc}	4,67 ± 0,05^{c}	3,73 ± 0,02^{a}	4,43 ± 0,49^{ab}
A_3B_2	4,26 ± 0,05^{ab}	4,78 ± 0,02^{b}	3,71 ± 0,03^{a}	4,65 ± 0,12^{a}
A_3B_3	4,15 ± 0,07^{ab}	4,87± 0,02^{a}	3,72 ± 0,02^{a}	4,67 ± 0,12^{a}

The results showed that the A1B3 and A3B2 treatments gave the highest scores for the colour parameters of 4.31 ± 0.02 and 4.26 ± 0.05, respectively, with a statistically significant difference ($p < 0.05$). The preferred colour of the bolu cukke is related to the brightness and harmony between the sponge's natural colour and the fish bones' colour. Fortification of fish bone powder in the A2B3 and A3B3 treatments lowered the colour score. This is caused by an increase in mineral compounds such as calcium phosphate which can interfere with the visual appearance of bolu cukke. Based on research Hurrell (2022) This indicates that the optimal color in fortified food products is achieved when the ratio of additives to the main ingredient is not dominant.

The aroma parameter in bolu cukke was the highest value in the A3B3 treatment (high bone and high flavor) with a score of 4.87 ± 0.02, statistically very significant from other treatments ($p < 0.01$). Theoretically, this increase in aroma score can be explained by the volatile interaction between the aromatic compounds of tiramisu flavors (such as vanillin, ethyl maltol, and other phenolic compounds) and the protein and lipid compounds from the fish bones that can produce complex aromas during the roasting process. Based on research Dai et al. (2024) The flavor of tiramisu contains volatile compounds such as vanillin and esters that can mask the fishy aroma of the fish bones. The volatility of aromatic compounds increases with a Maillard reaction between amino acids and reducing sugars, which are reinforced in the dough containing bone protein. The closure of fishy odors by these flavors also strengthens the theory of flavor masking in food formulations [38]. Thus, strong-flavoured flavors such as tiramisu can neutralize the distinctive smell of animal protein in sweet bread products.

The results of observations on the texture value of bolu cukke. This study's highest average texture score was found in the treatment of A2B2, A3B1, A3B2, and A3B3, with values ranging from 3.68 to 3.73, although there was no statistically significant difference ($p > 0.05$). These results show that the variation in calcium fortification levels and flavor addition did not significantly affect the sensory value of the texture of bolu cukke sponge. Particle size and homogenization process are the main factors in maintaining texture consistency. Fish bone powder functions as a filler that can strengthen the structure of protein-starch gel during the baking process [39]. So, fish bone-based fortification materials need to be controlled in micro or nano form to maintain the softness of bakery products.

Taste is a complex sensory parameter involving basic taste, aroma, and texture. In this study, the flavour of tiramisu not only plays a role in masking the fishy taste of fish bones but also enriches the flavour profile through the sweet and creamy compounds typical of tiramisu products. The roasting process also gives rise to additional flavour compounds from the protein-fat interaction derived from the fish bones, through the Maillard reaction and caramelisation mechanism, resulting in a roasty taste that contributes positively to the panellists' preferences. The flavour of tiramisu successfully masks the fishy flavor of the fish's bone calcium, which is commonly found in fish-based fortification studies [40,41].

Overall, treatments combining tiramisu flavour and calcium fortification tended to have optimal concentrations, resulting in the highest organoleptic scores, especially in aroma and flavour parameters. This combination synergises calcium fortification's nutritional value and tiramisu flavour's sensory value. The interaction between tiramisu flavour and calcium fortification had a noticeable effect on aroma and taste ($p < 0.05$), but not on texture. These results indicate that chemical-based sensory aspects (aroma and taste) are more sensitive to formulation variations, while texture is more determined by the physical character of the dough. The change in colour and aroma in the fortified bolu cukke is not only related to the panellists' perception, but it can also be explained that the darker or brownish colour arises from the formation of the melanoidine compound due to the Maillard reaction between the amino acids of fish bones and sugar during baking. The complex aromatic compounds also contribute to the preferred special aroma. Adding tiramisu flavors enriches the spectrum of volatile compounds, creating a more thorough and complex sensory experience. In general, the results of this study support but also expand on the results of previous studies. Based on research Settapramote et al. (2024) States that fish bone fortification can lower organoleptic scores due to tastes and aromas that consumers less accept. However, in this study, tiramisu flavor neutralised the negative characteristics of fish bones.

4-CONCLUSION

Enzymatic extraction using bromelain from pineapple peels effectively recovers calcium from Threadfin Bream fish bones, yielding 43.8% extract with 28.5% calcium content. Fortifying *bolu cukke* with this calcium source improves its physicochemical properties and maintains acceptable sensory quality, especially with 30% calcium and 45% tiramisu flavoring. The findings highlight the potential of fish bone waste as a sustainable calcium source for functional foods, supporting marine-based food diversification and enhanced community calcium intake.

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6-REFERENCES

[1] Cheng L, Lian J, Ding Y, Wang X, Munir MAM, Ullah S, et al. Calcium deficiency and its implications for cardiovascular disease and cancer: Strategies for resolution via agronomic fortification. Food Sci Nutr 2024;12:8594–607. https://doi.org/10.1002/fsn3.4464.

[2] Jin X, Jin X, Guan W, Tang M. Dietary Calcium-to-Phosphorous Ratio, Metabolic Risk Factors and Lipid Accumulation Product, Skeletal Muscle Mass, and Visceral Fat Area Among Healthy Young Individuals. Int J Sport Nutr Exerc Metab 2025;35:43–50. https://doi.org/10.1123/ijsnem.2024-0062.

[3] Khairul UT, Idris NIM, Shah RM, Nawi IHM, Che Soh N. Evaluation of Minerals Composition in Fish Bone Meal as Organic Fertilizer Development for Sustainable Environment. Current World Environment 2025;19:1260–8. https://doi.org/10.12944/CWE.19.3.17.

[4] López-Álvarez M, Souto-Montero P, Durán S, Pérez-Davila S, Vázquez JA, González P, et al. Valuable Ca/P Sources Obtained from Tuna Species' By-Products Derived from Industrial Processing: Physicochemical and Features of Skeleton

Fractions. Recycling 2024;9:109. https://doi.org/10.3390/recycling9060109.
[5] Manchanda M, Rawat D, Chandra A, Saini RK. Development and Evaluation of Calcium-Fortified Multi-Millet Biscuits: A Nutritious Alternative to Refined Wheat Flour. Foods 2024;13:1696. https://doi.org/10.3390/foods13111696.
[6] Novianty H, Sefrienda ARSR, Jasmadi J. Analyzing the Characteristics of Fishbone Powder Derived from Pangasius sp., Thunnus tonggol, and Thunnus albacares as Food Fortificant. AgriTECH 2024;44:90. https://doi.org/10.22146/agritech.79972.
[7] Pérez A, Ruz M, García P, Jiménez P, Valencia P, Ramírez C, et al. Nutritional Properties of Fish Bones: Potential Applications in the Food Industry. Food Reviews International 2024;40:79–91. https://doi.org/10.1080/87559129.2022.2153136.
[8] Fitri M, Tartar SU, Nurmiah S, Syukroni I. Degradation of Milkfish Bone (Chanos- chanos Forsskal) with Pure Papain Enzyme Concentration and Heating Time. Asian Food Science Journal 2022:78–85. https://doi.org/10.9734/afsj/2022/v21i12607.
[9] AOAC. Official Methods of Analysis of The Association of Official Analytical Chemist 18th Edition. Gaithersburg, USA: AOAC International; 2005.
[10] Huang Y-C, Hsiao P-C, Chai H-J. Hydroxyapatite extracted from fish scale: Effects on MG63 osteoblast-like cells. Ceram Int 2011;37:1825–31. https://doi.org/10.1016/j.ceramint.2011.01.018.
[11] Sriuttha M, Chanshotikul N, Hemung B. Calcium extraction from catfish bone powder optimized by response surface methodology for inducing alginate bead. Heliyon 2024;10:e30266. https://doi.org/10.1016/j.heliyon.2024.e30266.
[12] Antu MstAR, Ali MS, Ferdous MJ, Ahmed MdT, Ali MdR, Suraiya S, et al. Recovery and Characterization of Calcium-Rich Mineral Powders Obtained from Fish and Shrimp Waste: A Smart Valorization of Waste to Treasure. Sustainability 2024;16:6045. https://doi.org/10.3390/su16146045.
[13] Guo J, Zhu S, Chen H, Zheng Z, Pang J. Ultrasound-assisted solubilization of calcium from micrometer-scale ground fish bone particles. Food Sci Nutr 2022;10:712–22. https://doi.org/10.1002/fsn3.2696.
[14] Malde MK, Bügel S, Kristensen M, Malde K, Graff IE, Pedersen JI. Calcium from salmon and cod bone is well absorbed in young healthy men: a double-blinded randomised crossover design. Nutr Metab (Lond) 2010;7:61. https://doi.org/10.1186/1743-7075-7-61.
[15] Liu X, Wei X, Skibsted LH, Tomasevic I, Yao X, Wang W, et al. Investigation of the peptides with calcium chelating capacity in hydrolysate derived from spent hen meat. J Food Sci 2024;89:2277–91. https://doi.org/10.1111/1750-3841.17023.
[16] Taufiq Nuramaniyah, Fadlila Risky Nurul. Pembuatan Nano Partikel Kalsium (Ca) dari Limbah Tulang Ikan Patin (Pangasius sp) Menggunakan Metode Ultrasound- Asissted Solvent Extraction. Al-Kimia 2021;9:9–15. https://doi.org/https://doi.org/10.24252/AL-KIMIA.V9I1.16390.
[17] Marasabessy I, Sudirjo F, Nara S. Karakteristik Tepung Tulang Ikan Pelagis dan Demersal sebagai Sumber Kalsium. Jurnal Ilmiah Inovasi 2019;18:133–6. https://doi.org/10.25047/jii.v18i3.1241.
[18] Alshemary AZ, Cheikh L, Çardaklı İS. Extraction and degradation rate analysis of calcium phosphate from diverse fish Bones: A comparative study. Journal of Saudi Chemical Society 2024;28:101859. https://doi.org/10.1016/j.jscs.2024.101859.
[19] Guo J, Zhu S, Chen H, Zheng Z, Pang J. Ultrasound-assisted solubilization of

calcium from micrometer-scale ground fish bone particles. Food Sci Nutr 2022;10:712–22. https://doi.org/10.1002/fsn3.2696.
[20] Zhang J, He S, Kong F, Huang S, Xiong S, Yin T, et al. Size Reduction and Calcium Release of Fish Bone Particles During Nanomilling as Affected by Bone Structure. Food Bioproc Tech 2017;10:2176–87. https://doi.org/10.1007/s11947-017-1987-z.
[21] Eknapakul T, Jiamprasertboon A, Amonpattaratkit P, Pimsawat A, Daengsakul S, Tanapongpisit N, et al. Unraveling the structural complexity of and the effect of calcination temperature on calcium phosphates derived from Oreochromis niloticus bones. Heliyon 2024;10:e29665. https://doi.org/10.1016/j.heliyon.2024.e29665.
[22] Zhu L, Liu H, Witkowska HE, Huang Y, Tanimoto K, Li W. Preferential and selective degradation and removal of amelogenin adsorbed on hydroxyapatites by MMP20 and KLK4 in vitro. Front Physiol 2014;5. https://doi.org/10.3389/fphys.2014.00268.
[23] Wijayanti I, Sookchoo P, Prodpran T, Mohan CO, Aluko RE, Benjakul S. Physical and chemical characteristics of Asian sea bass bio-calcium powders as affected by ultrasonication treatment and drying method. J Food Biochem 2021;45. https://doi.org/10.1111/jfbc.13652.
[24] Jindal A, Patil N, Bains A, Sridhar K, Stephen Inbaraj B, Tripathi M, et al. Recent Trends in Cereal- and Legume-Based Protein-Mineral Complexes: Formulation Methods, Toxicity, and Food Applications. Foods 2023;12:3898. https://doi.org/10.3390/foods12213898.
[25] Paramita BL, Sahubawa L, Ratnawati SE. Physicochemical Properties and Sensory Evaluation of Wheat-Mocaf-Based Dried Noodles Enriched with Catfish Bone Flour (Clarias sp.). Indonesian Food and Nutrition Progress 2022;18:60. https://doi.org/10.22146/ifnp.68032.
[26] Rocha RAR, Ribeiro MN, Silva GA, Rocha LCR, Pinheiro ACM, Nunes CA, et al. Temporal profile of flavor enhancers MAG, MSG, GMP, and IMP, and their ability to enhance salty taste, in different reductions of sodium chloride. J Food Sci 2020;85:1565–75. https://doi.org/10.1111/1750-3841.15121.
[27] Wajs J, Brodziak A, Król J. Shaping the Physicochemical, Functional, Microbiological and Sensory Properties of Yoghurts Using Plant Additives. Foods 2023;12:1275. https://doi.org/10.3390/foods12061275.
[28] Hashem AMA, Sakhare SD, Kudre TG. Effect of Piaractus brachypomus fish protein hydrolysate on physicochemical, sensory, and storage properties of cookies. Biocatal Agric Biotechnol 2023;51:102761. https://doi.org/10.1016/j.bcab.2023.102761.
[29] Salinas MV, Zuleta A, Ronayne P, Puppo MC. Wheat Flour Enriched with Calcium and Inulin: A Study of Hydration and Rheological Properties of Dough. Food Bioproc Tech 2012;5:3129–41. https://doi.org/10.1007/s11947-011-0691-7.
[30] Liu H, Yang J, Xu Y, Wen J, Zhou J, Xu Z, et al. Effects of Glycerol Monooleate on Improving Quality Characteristics and Baking Performance of Frozen Dough Breads. Foods 2025;14:326. https://doi.org/10.3390/foods14020326.
[31] Zhuang Y, Wang Y, Yang H. Effect of cation valence on the retrogradation, gelatinization and gel characteristics of maize starch. Food Chem 2024;450:139307. https://doi.org/10.1016/j.foodchem.2024.139307.
[32] Yamakita E, Nakashima S. Water Retention of Calcium-Containing Pectin Studied by Quartz Crystal Microbalance and Infrared Spectroscopy with a Humidity Control System. J Agric Food Chem

2018;66:9344–52. https://doi.org/10.1021/acs.jafc.8b02413.
[33] Silva CR, Francisquini J d'Almeida, Stephani R, de Carvalho AF, de Oliveira LFC, Perrone ÍT. Effect of maltodextrin and inulin addition on the dairy-based powders properties. Drying Technology 2024;42:612–21. https://doi.org/10.1080/07373937.2023.2294352.
[34] Qu M, Jiang P, Zhu Y, Zhu X, Liu L, Huang Y. Effects of glutenin/gliadin ratio and calcium ion on the structure and gelatinity of wheat gluten protein under heat induction. Food Biosci 2024;58:103704. https://doi.org/10.1016/j.fbio.2024.103704.
[35] Di Rosa C, De Arcangelis E, Vitelli V, Crucillà S, Angelicola M, Trivisonno MC, et al. Effect of Three Bakery Products Formulated with High-Amylose Wheat Flour on Post-Prandial Glycaemia in Healthy Volunteers. Foods 2023;12:319. https://doi.org/10.3390/foods12020319.
[36] Hurrell RF. Ensuring the Efficacious Iron Fortification of Foods: A Tale of Two Barriers. Nutrients 2022;14:1609. https://doi.org/10.3390/nu14081609.
[37] Dai W, He S, Huang L, Lin S, Zhang M, Chi C, et al. Strategies to reduce fishy odor in aquatic products: Focusing on formation mechanism and mitigation means. Food Chem 2024;444:138625. https://doi.org/10.1016/j.foodchem.2024.138625.
[38] Chen L, Yang F, Jiang Q, Gao P, Xia W, Yu D. Effect of different starch on masking fishy odor compounds. Int J Biol Macromol 2024;268:131911. https://doi.org/10.1016/j.ijbiomac.2024.131911.
[39] Wei Y, Wang C, Guo X, Wang Z, Deng X, Zhang J. Enhancement of micron fish bone on gelling and 3D printing properties of Northern pike (Esox lucius) low-salt surimi. LWT 2024;208:116709. https://doi.org/10.1016/j.lwt.2024.116709.
[40] Desai AS, Beibeia T, Brennan MA, Guo X, Zeng X-A, Brennan CS. Protein, Amino Acid, Fatty Acid Composition, and in Vitro Digestibility of Bread Fortified with Oncorhynchus tschawytscha Powder. Nutrients 2018;10:1923. https://doi.org/10.3390/nu10121923.
[41] Damerau A, Mustonen SA, Ogrodowska D, Varjotie L, Brandt W, Laaksonen O, et al. Food Fortification Using Spray-Dried Emulsions of Fish Oil Produced with Maltodextrin, Plant and Whey Proteins—Effect on Sensory Perception, Volatiles and Storage Stability. Molecules 2022;27:3553. https://doi.org/10.3390/molecules27113553.
[42] Settapramote N, Kawee-ai A, Niyomsri P, Koyram W, Phungam N. Consistent effect of Nile tilapia (Oreochromis niloticus) fortification in biscuit on nutritional composition, consumer perception and shelf life properties. Food Res 2024;8:229–34. https://doi.org/10.26656/fr.2017.8(3).230.